

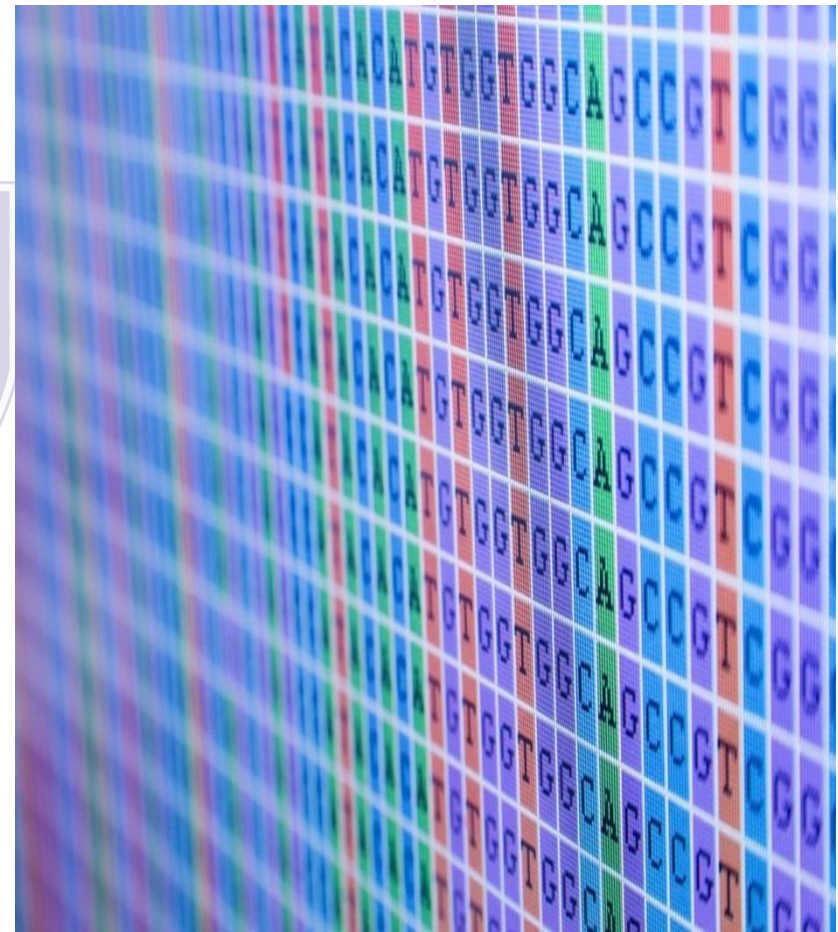


FACULTY OF ENGINEERING & TECHNOLOGY
DEPARTMENT OF BIOTECHNOLOGY

- What is DNA Sequencing ?



- History of development
- Basic Methods- Chain termination and Chemical modification method



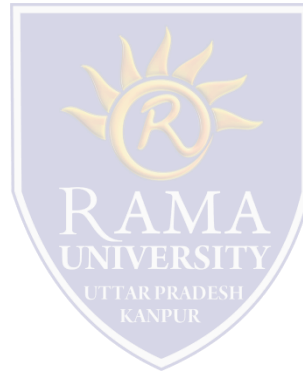
- Determining the precise order of nucleotides in DNA.



- We need to determine the order of nucleotide bases in a strand of DNA for sequencing.

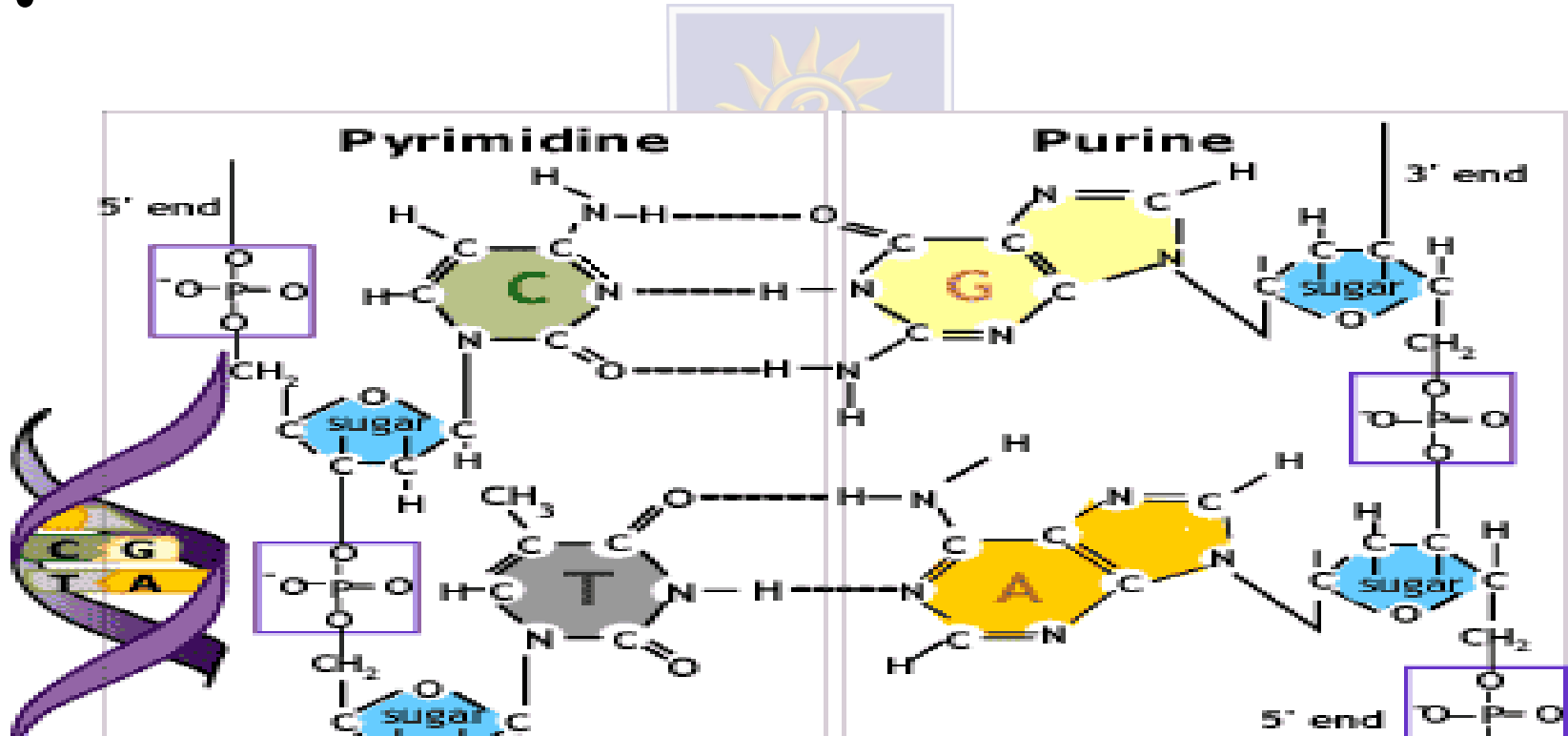
The Need for DNA Sequencing

- Gene isolation
- Sequence characterization
- Forensics
- Molecular Archeology
- Gene Gene Interaction
- Gene Protein Interaction
- Cloning



DNA

- Deoxyribonucleic Acid Stores genetic information
- Four different nucleotides A, T, G, C
- DNA comprises of a long molecule analogous to a chain, while the links of the chain are called Nucleotides
-



Historical Timeline

1870 – Miescher discovers DNA

1940 - Avery: Proposes DNA as ‘Genetic Material’ 1953 –

Watson & Crick “double helical structure” 1970 - Wu:

Sequences λ Cohesive End DNA 1977 – Sanger: Dideoxy

Chain Termination

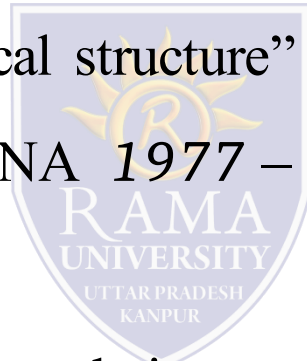
1977 – Gilbert: Chemical Degradation

1986 – Partial Automation

1990 – Cycle Sequencing, Improved Sequencing Enzymes,

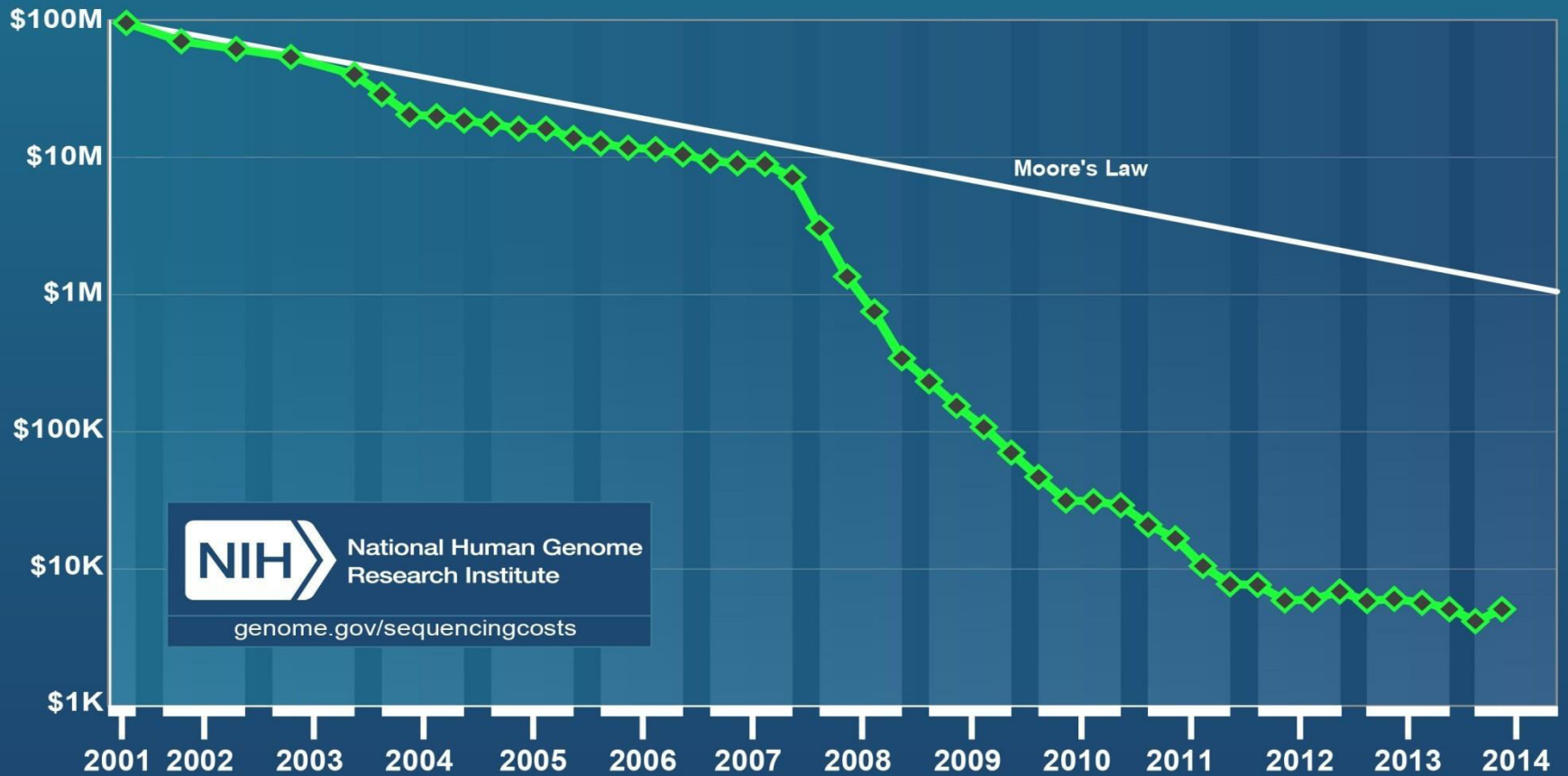
Improved fluorescent detection schemes

2002 – NGS: 454 , pyro sequencing



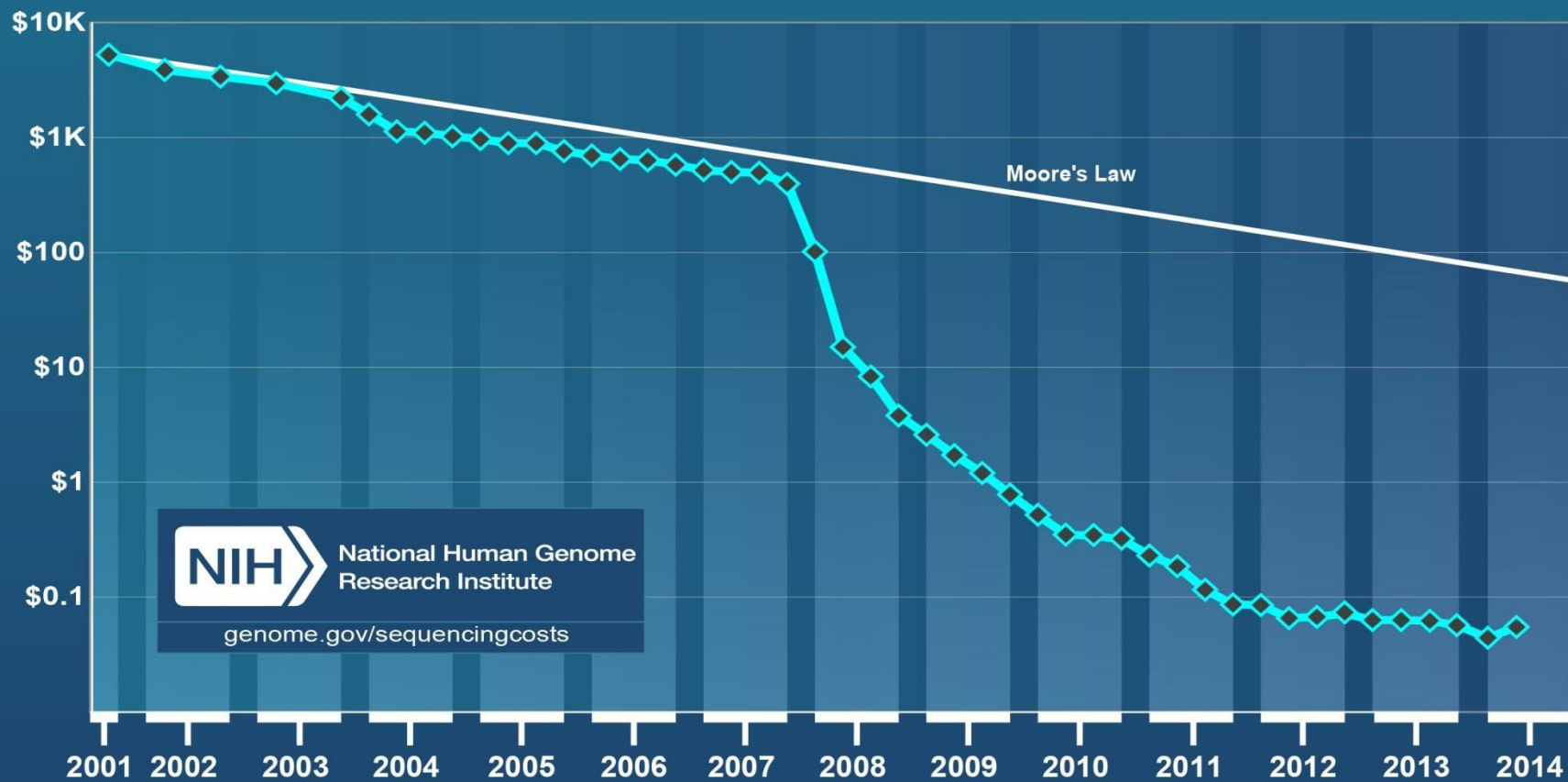
Cost per Genome

Cost per Genome



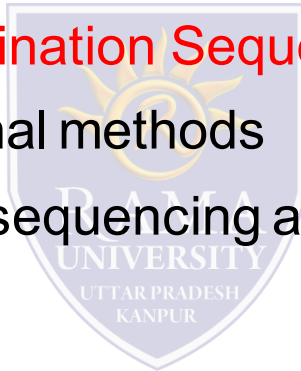
Cost per Megabases

Cost per Raw Megabase of DNA Sequence



Sequencing Methods

- To determine the order of the nucleotide bases adenine, guanine, cytosine, and thymine in a molecule of DNA two methods were used
 1. Maxam and Gilbert; Chemical Sequencing
 2. Sanger; Chain Termination Sequencing
- These two are conventional methods
- Robotics and automated sequencing are based on these methods



- In 1976–1977, Allan Maxam and Walter Gilbert developed a DNA sequencing method based on **chemical modification** of DNA and subsequent **cleavage** at specific bases
 - I. Chemical Modification of DNA; radioactive labeling at one 5' end of the DNA (typically by a kinase reaction using gamma-³²P ATP)
 - II. Purification of the DNA fragment to be sequenced
 - III. Chemical treatment generates breaks in DNA
 - IV. Run on the gel

